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Biogeosciences Discussions

Interactive comment on “Response of *Halimeda* to ocean acidification: field and laboratory evidence” *by* L. L. Robbins et al.

Anonymous Referee #1

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GENERAL COMMENTS

This study deals with the effects of a decrease in pH on the calcification of crystals (morphology and abundance) among different species of the calcareous green alga Halimeda, using scanning electron microscopy. The study comprises (a) results from a laboratory experiment comparing crystal shape and abundance of two species of Halimeda (H. opuntia and H. tuna) maintained at pH 8.1 and pH 7.5 and (b) observations of crystal shape and abundance changes over 40 years (from 1966-1967 and 2008) in two other species of Halimeda (H. incrassata and H. discoidea) obtained from archived samples. The authors showed a shift in crystal size and amount between pH: an increase in crystal concentration and a decrease in crystal width with decreasing pH. They also showed similar changes over a 40 year time period with an increase in

crystal concentration and a decrease in crystal width from 1960s to 2008. The authors explained this previous change by a decrease in pH over the last 40 years.

While the main objective of this study, which is to combine both field and laboratory observations, is really interesting, major issues remain: (1) different species were used for historical data observations and laboratory experiment, (2) variations in pH tested in laboratory (Δ = 0.6 pH units, pH 7.5 and 8.1,) do not reflect those from the past 40 years (Δ<0.1 pH unit), (3) there is no information on the environmental parameters, especially on the seawater carbonate chemistry, of the sampling sites and at the time of algal collection (ie. present and archived samples), and (4) there is no evaluation of the intravariability of crystal morphology and density in the sampling area of the west Florida shelf.

(1) Why did the authors use different species for the field work and the laboratory experiment? The authors showed that Halimeda species present species specific differences in responses to pH changes: number and shape of crystals drastically increased and decreased, respectively, with decreasing pH, for H. opuntia, while these morphological parameters remained similar between pH for H. tuna. In this study, there was no laboratory investigation on the effects of pH on H. incrassata and H. discoidea (archived samples). Accordingly, from this study, we don’t know if pH influence the calcification of these species. It is thus not possible to conclude if the morphological changes observed over the 40 year time period are due to pH or to an other environmental parameter.

(2) One of the objectives of this study was to investigate the effects of pH between 1960s and 2000s. The decrease in pH over these last 40 years was very low. According to the shift in pH over the last 200 years of 0.1 pH units, the pH variation between 1966-67 and 2008 in the studied area is likely to be less than 0.1 pH units. Why did the authors choose an extreme pH value of 7.5 rather than a value of 8.2 to test their hypothesis? The variation of pH of 0.6 units (from 8.1 to 7.5) tested in the laboratory study is consequently far from that in the field over the last 40 years (< 0.1 pH units).

As a consequence, it is difficult to understand how calcification response to less than 0.1 pH unit change (historical records; 48 to 70% crystal mean width decrease and 39-62% abundance increase) can be more drastic than the response to a shift of 0.6 pH unit (laboratory experiment, 18 to 43% crystal mean width decrease and 11-65% abundance increase). This point is likely to justify that crystal concentration and morphological changes in the field may be related to other environmental changes than pH.

(3) I profoundly regret that the authors could not provide any information on the environmental parameters for the present and past sampling sites in order to provide a more robust test of their hypotheses. What was the evolution of seawater pH in the studied area over the last 40 years? What about the other environmental parameters, which can influence algal calcification, such as temperature, irradiance, seawater chemistry (total alkalinity, nutrient concentration, etc…), physical parameters (wave exposition, flow rate, etc…)? What are the effects of these factors on crystal morphology and density? Additionally, archived samples of H. discoidea used for comparison between recent and past effect of pH were collected at different season: ie. February for the year 2008 and December and August for the years 66-67. Is there any effect of seasonal pH fluctuations or other seasonal environmental changes on the concentration and morphology of crystals in the young apical segments that can develop in a few weeks?

(4) Before testing the effects of time on crystal calcification, the authors should have tested the influence of the other environmental parameters. They should have also tested the homogeneity in algal crystal morphology and concentration in the sampling area of the west Florida shelf

I suggest to the author to find and provide data in terms of carbonate chemistry and other environmental parameters in their study site from the last 40 years and to try to correlate their algal calcification results with each environmental parameters. I agree that their “approach provides a tool for predicting future changes” but it is pure spec-

ulation to write that “this approach allows evaluation of how ocean acidification has affected green alga” (Introduction, P. 4898, L. 27-28).

Moreover, this paper presents major flaws in the methodology, the results and the discussion sections. There is no information on the parameters of seawater carbonate chemistry, except pH. There was no replicate of culture conditions, only one aquarium at pH 7.5 and an other one at pH 8.1 (pseudoreplication). There is no information on the statistical test used to compare calcification results between sites, dates or pH. In addition, there is an absence of a constructive discussion based on the results: the two first paragraphs of the discussion sevtion present a synthesis on Halimeda calcification. Only the third one, limited to 9 lines, is a discussion of the results.

SPECIFIC COMMENTS

Title

The content of the manuscript does not justify the title “Response of Halimeda to ocean acidification: field and laboratory evidence”. I agree that the authors showed evident relationships between pH and Halimeda crystal calcification in the laboratory work but the morphological changes observed in the field samples are only dependent on time. The authors do not provide any information on seawater pH (or pCO2) at the sampling sites and the dates of algal collection; accordingly there is no field evidence that Halimeda responds to ocean acidification, this remains a hypothesis but can not be considered as an evidence.

Abstract

P.4896, L.2: delete "In fact".

Introduction

Reduce the part on “Halimeda response” which is disproportionate in comparison with the rest of the text and include it in a unique Introduction part. Be more focused on the objectives of this study and present the hypotheses tested.

P. 4897, L. 26: Delete fig 1. Picture of Halimeda to illustrate the purpose is useless.

P. 4898, L. 24-27: “Our study… with laboratory experiment to simulate predicted carbonate chemistry ….in the future”. Replace “carbonate chemistry” by “pH”: the experiment do not simulate predicted carbonate chemistry but only predicted pH since there is no information on the other parameters of the carbonate chemistry (total alkalinity for example).

P. 4898, L. 27-28: I agree that this “approach provides a tool for predicting future changes” but not that “this approach allows evaluation of how ocean acidification has affected green alga” over the last 40 years. Delete this part of the sentence.

P. 4899, L. 2: Which samples were from 1980s? There is no reference to these samples in the Mat & Met and results sections.

Material and methods

P. 4899, L. 10-14: Where the archived samples exactly come from? Specify and give geographic coordinates. Since there is a strong interlocality variability, explain why the locations of the samples were not exactly the same: ie. “Notably, we used samples from the west Florida shelf, off Tampa Bay”. Give information on the distance between sampling sites in this bay. Are the environmental parameters homogenous in this bay?

P. 4899, L. 10: Give the names of the species of archived Halimeda samples.

P. 4899, L. 17: Delete Fig. 2 and replace it by the exact geographic coordinates.

P. 4899, L. 17: Which species are “purchased from a dealer”, H. tuna, H. opuntia or both? Did you have accurate information on the site where the dealer collected the samples? Specify the accurate date of collection both for samples collected in the field and those purchased from a dealer.

P. 4899, L. 17: Why did the authors used different species in the laboratory study (H. tuna and H. opuntia) and in the field study (H. incrassata and H. discoida). Where the

two previous species absent at the time of collection? The fact that different species are used in the laboratory study “because of their availability” (P. 4899, L. 18) is not a good argument to justify the choice of the species.

P. 4899, L. 18-19: How could you affirm that “the specimens were obtained from sites with pH 8.1”? Did you measure pH? Provide more accurate information on the pH of the sampling site (pH scale, pH variability).

P. 4899, L. 21-22: Why did the authors present “only the apical data” in this paper?

P.4900, L. 2: Why did not the authors use natural seawater from the study site but “artificial seawater”? Is it an open system or close system? What is the seawater renewal rate in the tanks?

P.4900, L. 3: Delete Fig. 3. The picture of laboratory experiments already described in the text is useless.

P.4900, L. 3: The aragonite sand substrate contained in the tank can influence carbonate chemistry of seawater in the tanks, especially total alkalinity. The aragonite sand is likely to dissolve faster at pH 7.5 than at pH 8.1 and to cause differential total alkalinity values in the tanks. Accordingly the authors must provide total alkalinity values in their tanks. The calcification of their algae may be profoundly affected by seawater alkalinity.

P.4900, L. 7 and 11-12: Specify the variability of pH associated with the mean values of pH 7.5 and 8.1. Were pH values continuously recorded?

P.4900, L. 7 and 15-16: Precise which buffers were used for total hydrogen ion concentration pH scale? Clarify and justify the use of two different calibrations, this is unclear in the text.

P.4900, L. 7 and 18-19: The calibration accuracy was confirmed according to Dickson et al. (2007), did you used Dickson standards? Did the authors apply all the recommendation provided by Dickson et al. for spectrophotometric measurements: ie. high quality spectrophotometer, temperature control system for spectrophotometer cell, thermostated bath to within ±0.05°C, etc. . . Be more specific.

P. 4901, L. 6: Precise which version of Image J software?

P. 4901, L. 6: Justify the use of different size of area (10×10 μm and 5×5 μm) to count the crystals.

P. 4901, L. 9: Delete the name of the functions used in the software (ie. "measuring tool").

Results and discussion

Provide two distinct parts.

Provide information on statistical tests performed to compare calcification data between sites, dates and pH! Are the differences ("differences were observed", "demonstrated a decrease", "showed a trend", etc...) significant or not?

P. 4902, L. 7: Delete "for example".

P. 4902, L. 13: Replace "Fig. 5 a-d" by "Fig. 5".

P. 4902, L. 16-17: Present the results from 1990 and 2000 directly on Fig. 6. Provide standard errors associated to the means in the text.

Discussion

The two first large paragraphs are only a bibliographic synthesis on Halimeda calcification without any direct relationship with the results. Only the third paragraph (9 lines) discusses the results obtained in this study.

P. 4903, L. 16: Delete Fig. 10, unuseless.

P. 4904, L. 22: Replace "pCO2" by "pH" since there was no measurements pCO2 in this study.

Interactive comment on Biogeosciences Discuss., 6, 4895, 2009.